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金沙江河谷特有植物罂粟莲花的保护遗传学*

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摘要: 毛茛科的罂粟莲花 (*Anemoclema glaucifolium*) 是中国西南部金沙江河谷地带特有的单种属植物。本研究利用分子生物学手段 (SNPs), 对罂粟莲花的遗传多样性和遗传结构进行了研究。三个叶绿体片段 (*rps*16 内含子, *psb*A-*trn*H 基因间隔区以及 *trn*C-*ycf*6 基因间隔区) 联合分析的结果显示低的遗传多样性和高的遗传分化。这可能是由于居群间长期的地理隔离, 狭小的分布区以及生境片段化造成的有限的基因流所引起的。如今在金沙江上修建水电站的位置与罂粟莲花的分布区有部分重叠, 这些水电站会淹没罂粟莲花的部分个体和生境, 并且改变生态环境, 威胁罂粟莲花的生存。本研究对罂粟莲花遗传多样性的研究为制定有效的保护策略提供了信息。

关键词: 罂粟莲花; 叶绿体 DNA; 单核苷酸多样性; 遗传多样性; 保护遗传学
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Conservation Genetics of an Endemic Plant, *Anemoclema glaucifolium*, in the Jinsha River Valley

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Abstract: *Anemoclema* W. T. Wang, a monotypic genus of Ranunculaceae, is endemic to the Jinsha River Valley in southwest China. Combining field investigation with molecular marker analysis (SNPs), we investigated the genetic diversity and genetic structure of *A. glaucifolium*. Analysis of three chloroplast DNA (cpDNA) regions (*rps*16 intron, *psb*A-*trn*H intergenic spacer and *trn*C-*ycf*6 intergenic spacer) revealed a low level of genetic diversity within the species, but high divergence among populations. This genetic structure is possibly caused by a long period of historical geographic isolation, a relatively narrow distribution range and limited gene flow due to habitat fragmentation. Hydropower stations are scheduled to be built in the Jinsha River drainage system in areas that overlap the range of *A. glaucifolium* and as a result of their construction the habitats of *A. glaucifolium* will be flooded or adversely affected in other ways, thus threatening the survival of the species. The results of our analysis of genetic diversity in *A. glaucifolium* are of value for developing an appropriate conservation strategy for this vulnerable species.

Key words: *Anemoclema glaucifolium*; cpDNA; SNPs; Genetic diversity; Conservation genetics

Jinsha River Valley locates at the basal zone of the Hengduan Mountains region, which is one of the 25 global biodiversity hotspots as featuring exceptional concentrations of endemic species (Myers *et*

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al., 2000). It is a special and vulnerable ecosystem characterized by aridity, high temperatures, semi-savanna vegetation and relatively few plant species (Jin *et al.*, 1994). However, there are especially rich for endemic genera (e. g., *Anemoclema*, *Nouelia*, *Musella*, *Ostryopsis*, *Trailliaedoxa*) and species (e. g., *Munronia delavayi*, *Aristolochia delavayi*, *Vitex dulouxii*, *Cotinus nana*, *Mastixia microcarpa*) (Jin *et al.*, 1994). Due to natural, historical and anthropogenic factors, this ecosystem has become seriously degraded, which has resulted in soil and water losses and significant difficulties in vegetation restoration.

Jinsha River, the upper part of the Changjiang River, is one of the largest rivers in southwest China, which originates from the Qinghai-Tibet plateau. A total of 14 large-scale hydropower stations are planning to be built along Jinsha River drainage, of which, some have been building, such as, Xiangjiaba hydropower station, Xiluodu hydropower station and so on. The construction of hydroelectric power stations along Jinsha River will cause the rise of water level, so that various types of ecosystem would suffer serious destruction, and vertical climate would disappear. Moreover, many species would extinct owing to the submerged habitat in Jinsha River Valley. Firstly, the habitat of some species would be submerged, so that these species, especially endemic species, might extinct. For example, the construction of the Three Gorges Dam caused the native area of *Myricaria laxiflora* to be submerged. Fortunately, *ex situ* conservation of *M. laxiflora* in advance preserved some individuals (Wu *et al.*, 1998; Wang *et al.*, 2003). Secondly, various ecological types would be destroyed after dams retained water. Vertical climate would be changed, especially the temperature and humidity of the valley. This change would threaten the existence of species that had adapted to dry-hot or dry-warm environment. Last, there would be some direct influence on species in construction area. The original vegetation covered area would be occupied by construction and living facilities, so that the area might lose ability to regulate the ecological environment.

If there is no effective measures to conduct conservation strategy and population reconstruction, large-scale construction of hydropower stations along Jinsha River would cause some species to extinct in a short time, especially those populations close to the river. Studies show that before implementing conservation measures, genetic diversity and genetic structure of the species must be studied and potential benefits and risks of conservation strategy must be analyzed. Results of Marshall and Brown's study show that in order to preserve species and maintain normal evolution, more than 95% of the genetic diversity must be preserved (Marshall and Brown, 1975).

Anemoclema W. T. Wang, a monotypic genus of Ranunculaceae, is endemic to Jinsha River Valley in southwest China (Zhang and Gong, 2002). *Anemoclema glaucifolium* (Franch.) W. T. Wang is perennial herb, 45–80 (–150) cm tall. The blossom is in July to September (Liu, 1980) (Fig. 1). *A. glaucifolium* grows on the rock slope of Jinsha River Valley, where the climate is dry and hot or warm and the altitude ranges from 1 500 m to 3 000 m. As the common character species of this area, it is valuable in the study of phylogeny and adaption to specific environment of plants (Jin, 2002). Due to high ornamental value, *A. glaucifolium* may be cultivated as ornamental or act as breeding materials after being acclimated. Because *A. glaucifolium* is a monotypic genus species, there are rare relevant researches except a few studies about molecular systematics and cytology (Zhang and Gong, 2002; Wang *et al.*, 2009).

Considering from the level of the state to develop Jinsha River's water resources, it is urgent to protect threatened species. Detailed analysis of the levels and spatial distribution of genetic diversity is important for the development of effective conservation strategies and management practices for endangered species (Hedrick and Miller, 1992). Therefore, a conservation genetics research of *A. glaucifolium*

before construction of hydropower stations on Jinsha River is indispensable. The research explored the distribution of *A. glaucifolium* by field investigation and sampling, and studied the genetic diversity and genetic structure through SNPs (Single Nucleotide Polymorphisms). Then we could elaborate genetic background and the distribution of genetic variation within and among populations, so that effective conservative strategy can be made. The research can not only protect the endemic species *A. glaucifolium*, but also provide experience and conservative measures to protect other biological group in this area.

Materials and methods

Population sampling

In our study, leaf tissue was collected from 133 individuals, representing 7 populations of *A. glaucifolium* from its distribution range along Jinsha River during July in 2008 to September in 2009. All populations were collected in Yunnan Province, except for population QS, which was collected in Sichuan province. Information about each sampling location is presented in Fig. 2 and Table 1. Fresh and healthy leaves were dried with silica gel and stored at 4 ℃.

Fig. 1　*Anemoclema glaucifolium* in wild. (A) The leaves of *A. glaucifolium*; (B) The flower of *A. glaucifolium*

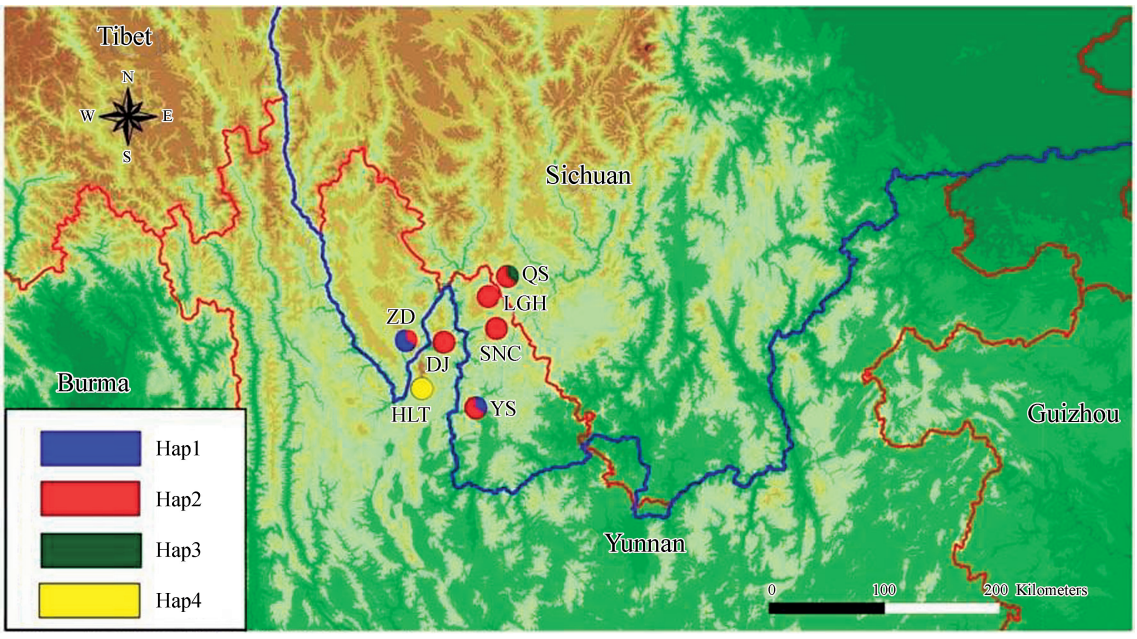

Fig. 2　Map showing the distribution of cpDNA haplotypes in the species. The pie sizes of sampled populations are proportional to their sample sizes

Table 1 Information of sampled populations of *A. glaucifolium*

	Population Code	Locality	Sample size	Latitude(N) /°	Longitude(E) /°	Altitude /m	Haplotypes (no. of individuals)
1	QS	Qiansuo, Yanyuan, Sichuan	29	28.340	101.258	2 650	Hap2(2), Hap3(1)
2	YS	Around Yongsheng, Yunnan	10	27.374	100.726	2 150	Hap1(1), Hap2(2)
3	SNC	Shuinichang, Ninglang, Yunnan	26	27.724	101.365	2 390	Hap2(4)
4	LGH	Luguhu, Ninglang, Yunnan	12	28.083	101.150	2 700	Hap2(3)
5	HLT	Xiangshan, Lijiang, Yunnan	24	27.399	100.517	2 530	Hap4(3)
6	DJ	Daju, Yulong, Yunnan	17	27.799	100.484	1 860	Hap2(3)
7	ZD	Hutiaoxia, Xianggelila, Yunnan	24	27.699	100.217	2 300	Hap1(2), Hap2(1)

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted following the CTAB protocol (Doyle and Doyle, 1987). After preliminary screening of a range of nucleic and organelle DNA, we chose three cpDNA fragments (*rps*16、*psbA-trn*H、*trn*C-*ycf*6) for the full survey because they contained the highest number of polymorphic sites.

PCR amplification was carried out in a 20 μL reaction volume, containing 30 ng template DNA, 2.0 μL 10×*Taq* Buffer (25 mM), 1.5–1.6 μL $MaCl_2$ (25 mM), 1.2–1.5 μL dNTPs (2.5 Mm), 1.0 μL dimethyl sulfoxide (10 μM), 0.3–0.35 μL each primer (10 μM), 1.5 unit of *Taq* polymerase and double-distilled water. PCR was performed in a T1 thermocycler (Biometra, Göttingen, German), with an initial denaturation at 94 ℃ for 3 min, followed by 30–36 cycles of denaturation at 94 ℃ for 45 sec, annealing at 52–55 ℃ for 30 sec to 70 sec, extension at 72 ℃ for 50 sec to 1.5 min, and a final extension cycle of 7 min at 72 ℃. All PCR products were purified directly using a PCR product purification kit (Sangon, Shanghai). Purified PCR products were sequenced in both directions with the same primers for the amplification reactions, using a 3730xl DNA analyzer by Sangon Biotech (Shanghai) Co., Ltd.

Sequences were aligned using Clustal X version 1.83 (Thompson *et al.*, 1997) and manually adjusted using BioEdit 7.0.5 (Hall, 1999). Indels were treated as the single mutation as substitution (Caicedo and Schaal, 2004). DnaSP version 4.10 (Rozas *et al.*, 2003) was used to calculate: (1) the number of haplotypes and variable sites; (2) nucleotide diversity per site (π) (Nei and Li, 1979); (3) haplotype diversity (H_d) (Nei and Tajima, 1983). Within-population diversity (H_S), total diversity (H_T), and two measures of population differentiation G_{ST} and N_{ST} were calculated using the HAPLONST. Program Arlequin version 3.1 (Excoffier *et al.*, 2005) was used to conduct an analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) and thus to estimate genetic variations within and among populations. Genealogical haplotype networks (with 95% most parsimonious connection limits) were constructed using TCS version 1.21 (Templeton and Sing, 1993; Clement *et al.*, 2000). To infer possible demographic expansion of *A. glaucifolium*, mismatch distribution analysis, based on the sudden population expansion model (Rogers and Harpending, 1992) using the observed number of differences between pairs of haplotypes, was conducted with DnaSP.

Results

Nucleotide and haplotype diversity

The aligned cpDNA *rps*16 data matrix was 801 bp in length and contained two polymorphic sites that resulted in two haplotypes. For the *psb*A-*trn*H region, the aligned length was 354 bp, with three haplotypes derived from two polymorphic sites. Region *trn*C-*ycf*6 was 429 bp in length, producing two haplotypes with only one mutation.

The total length of the combined *rps*16、*psb*A-*trn*H、*trn*C-*ycf*6 was 1 584 bp long with four substitutions (84, C/A; 525, A/C; 972, T/G; 1453,

C/A) and one indel (1138). A total of four haplotypes (Hap1-Hap4) were identified when all the sequences were combined (Table 2). The nucleotide diversity (π) at the species level was 0.00053 and the total haplotype diversity (H_d) was 0.325. In the TCS network of cpDNA haplotypes, the most widely distributed haplotype (Hap2) was in the interior position. The other three haplotypes were in the tip position, originating from Hap2 with one to three mutational steps (Fig. 3).

Table 2　Variable sites and distribution of four haplotypes of *A. glaucifolium*

Haplotype	Variable sites				
	*rps*16		*psb*A-*trn*H		*trn*C-*ycf*6
	84	525	972	1138	1453
Hap1	C	A	T	–	C
Hap2	C	A	T	T	C
Hap3	C	A	T	T	A
Hap4	A	C	G	T	C

–: indicates deletion

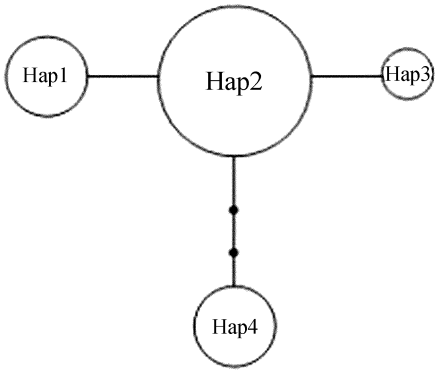

Fig. 3　Network of the four cpDNA haplotypes of *A. glaucifolium*. Hap1 to Hap4 represent haplotypes. The size of each circle is proportional to the haplotype frequency. Each solid line represents one mutational step that interconnects two haplotypes. The small bold circles indicate inferred intermediate haplotypes not detected in this investigation

Haplotype frequencies in each population and the distribution of the four haplotypes are presented in Table 1 and Fig. 2. Hap2 was widely distributed in every population except population HLT and fixed in population SNC, LGH and DJ. Hap1 occurred in population YS and ZD. Unique haploypes within population were detected in population QS and HLT.

Population analysis

On the population level, total diversity (H_T) was estimated to be 0.566 and within-population diversity (H_S) was 0.286 (Table 3). The comparison between N_{ST} and G_{ST} did not show a significant difference ($N_{ST}=0.435$, $G_{ST}=0.495$; $P>0.05$). For the total dataset, 90.31% of variation existed among populations, only 9.69% within populations, and a significant genetic differentiation was detected ($F_{ST}=0.90$) (Table 4).

Table 3　Genetic diversity and differentiation parameters based on the cpDNA fragment of *A. glaucifolium*

H_S	H_T	G_{ST}	N_{ST}
0.286 (0.1347)	0.566 (0.1527)	0.495 (0.2518)	0.435 (0.2866)

Standard errors are shown in parentheses

Table 4　AMOVA of the cpDNA fragment of *A. glaucifolium*

Source of variation	d. f.	SS	Variance components	Variation /%	Fixation Index (F_{ST})
Among populations	6	8.06	0.41	90.31	0.90 **
Within populations	15	0.67	0.04	9.69	
Total	21	8.73	0.45		

d. f. Degrees of freedom, SS sum of squares, ** $P<0.001$

Mismatch distribution analysis

The mismatch distribution based on the cpDNA haplotype dataset for the total samples was multimodal (Fig. 4) and inconsistent with the curve expected for an expanding population, indicating a demographic equilibrium ($P_{SSD}<0.01$).

Discussion

Genetic diversity

In this study, four haplotypes were found by analyzing conjointly three chloroplast non-coding areas (*rps*16 intron, *psb*A-*trn*H intergenic spacer and *trn*C-*ycf*6 intergenic spacer). The nucleotide diversity (π) is 0.00053 and the haplotype diversity

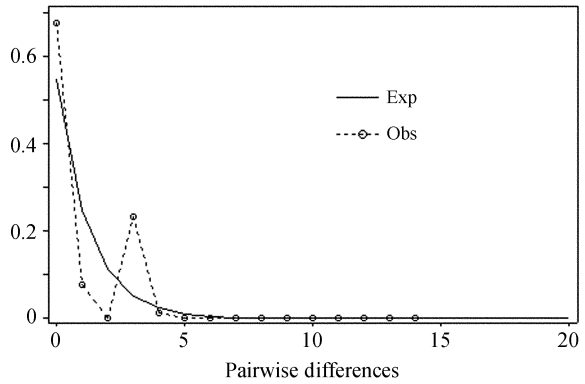

Fig. 4 Distribution of the number of pairwise nucleotide differences for cpDNA haplotypes in *A. glaucifolium*. The dashed line shows observed values, whereas the solid line represents expected values under a model of sudden (stepwise) population expansion

(H_d) is 0.325, both are lower compared with herb plants in the similar distribution area (Chen *et al.*, 2008). Genetic diversity is the consequence of long-term evolution. Its formation is the comprehensive result of sorts of factors, including current range, breeding system, seed dispersal mechanism and some historical causes like Quaternary glacial fluctuation, which can lead to repeated and drastic climate changes. All of these changes may cause bottleneck effect and founder effect, eventually, affecting distribution and genetic diversity of plants (Hamrick *et al.*, 1992). Unfortunately, information concerning pollination mechanisms and breeding system for this species is scarce. So we deduce the low genetic diversity of *A. glaucifolium* is the comprehensive result of limited individuals and some historical cause. Low average population genetic diversity ($H_S = 0.286$) was also detected. The reason is that there is only one haplotype in each four population out of the total seven populations, which also leads to high genetic differentiation among populations ($G_{ST} = 0.495$, $N_{ST} = 0.435$).

Genetic structure

Concerning the geographic distribution of haplotypes, the highest frequency Hap2 was distributed in six populations except population HLT and was fixed in three populations (SNC, LGH and DJ). According to coalescent theory, the haplotype in the interior position likely represents the ancestral haplotype, haplotype in tip position evolve from the ancestors (Wakeley, 2008). Therefore, inferred from the TCS network of cpDNA haplotypes (Fig. 4), we can conclude that the widely distributed Hap2 is ancestral haplotype and the other haplotypes (Hap1、Hap3 and Hap4) all evolve from Hap2. Hap1 and Hap3 originate from Hap2 with only one mutational step. Moreover, Hap1 and Hap2 coexist in population ZD and YS, while Hap2 and Hap3 coexist in population QS. These further illustrate the evolution relationship among Hap1, Hap3 and Hap2. In addition, the most southwestern population HLT fixes Hap4, which may result from geographical isolation or population expansion. But the mismatch distribution (Fig. 4) showed that a recent population expansion was unlikely in this case, so the unique Hap4 fixed in population HLT may be the consequence of long-term geographical isolation.

AMOVA showed that 90.31% of genetic variation existed among population while variation within population only accounted for 9.69% of the whole variation. In addition, we detected high level of genetic differentiation ($F_{ST} = 0.90$), much higher than the statistics studied by Petit (1999), which showing average value of genetic markers of 97 plant ($F_{ST} = 0.70$). Generally, species in fragmented habitat tend to have lower population genetic diversity and high genetic differentiation (Young *et al.*, 1996), which is in accordance with our results. Seven populations of *A. glaucifolium* in this study were distributed in separate habitat of fragmentation. The landscape characteristics of distribution area in Hengduan Mountains, where high mountains and deep valleys are distributed alternately, block gene exchange among populations (Li and Li, 1993). Besides, seeds of *A. glaucifolium* do not have special structures that facilitate long distance distribution or attachment to the animals, so the seed dispersal ability is limited. According to formula $Nm = (1-G_{ST})/4G_{ST}$, we calculated the Nm of *A. glaucifolium* ($Nm = 0.255$), which is less than 1. Wright thought that

if the value of gene flow among populations is less than 1, limited gene flow is the main reason leading to the genetic differentiation of the species (Wright, 1931).

Implications for conservation

A. glaucifolium is an endemic species in Jinsha River Valley, which are in small numbers. The genetic diversity of *A. glaucifolium* is relative low with high genetic differentiation among populations. Small populations of rare and endangered species have higher risks of extinction than larger stable ones, especially when gene flow between populations is restricted (Frankham *et al.*, 2002). As small populations are more susceptible to the loss of genetic diversity caused by genetic drift and inbreeding, which reduces heterozygosity and the performance of various fitness related traits.

According to field investigation, in some populations (YS、LGH), not only the population size is small, but also the number of adult individuals is rare, showing *A. glaucifolium* is in dangerous situation of extinction. Moreover, the narrow distribution area and fragmented habitat result in limited gene flow among populations, so that genetic drift of this species occurred easily. Human activities also cause significant damages to *A. glaucifolium*, especially developing hydroelectric power in Jinshan River Valley. 1) The construction of hydroelectric power stations in Jinsha River Valley will change the local climate with greatly increasing the water surface area. On one hand, humidity will increase, which is harmful to *A. glaucifolium*. Because *A. glaucifolium* has adapted to drought environment. On the other hand, the daily and annual temperature ranges will narrow, which may alter the phenophase of *A. glaucifolium*. 2) The wider river will interfere with the exchange of genes between populations located on opposite sides of the Jinsha River. 3) The hydropower station construction process produces a large amount of waste, which will change the soil structure, influence the local ecological environment and affect local plant growth (Mu *et al.*, 2010). 4) The current available farmland will be flooded, so the wilderness, where *A. glaucifolium* may grow, will be cultivated as farmland leading to the loss of the habitat of *A. glaucifolium*. Therefore, it is urgent to conduct effective conservation strategy.

Modern conservation biology aims at protecting not only species and habitats, but also genetic diversity of extant species for its evolutionary potentiality (Caughley and Gunn, 1996). Protection of genetic diversity needs to carry out corresponding measures according to genetic differences among populations. As to the development of protection strategy, genetic uniqueness is the main reference to identify prior population to protect (Arthur *et al.*, 2004). In our study, *A. glaucifolium* showed relatively low genetic diversity and high differentiation among populations. Population QS, HLT and YS contribute most to the total genetic diversity and allelic richness, and Hap 4 in HLT is unique. Therefore, these three populations should be given priority of protection.

Taking into account current distribution and conservation status of *A. glaucifolium*, for population QS, HLT and YS, *in situ* conservation strategy would be of practical value. Haplotypes in population ZD is the same as YS. But population ZD is close to Jinsha River and will be affected by hydropower station construction. Therefore, it is necessary to conduct *ex situ* conservation in population ZD, and a new population could be reestablished in the location with similar habitat afterwards. The seeds from all populations can be collected and preserved in Germplasm Bank of Wild Species in Southwest China. For the rest populations, *in situ* conservation is recommended.

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References:

Arthur JL, Camm JD, Haight RG *et al.*, 2004. Weighing conservation objectives: maximum expected coverage versus endangered species protection [J]. *Ecological Applications*, **14** (6): 1936—1945

Caicedo AL, Schaal BA, 2004. Population structure and phylogeogra-

phy of *Solanum pimpinellifolium* inferred from a nuclear gene [J]. *Molecular Ecology*, **13** (7): 1871—1882

Caughley G, Gunn A, 1996. *Conservation Biology in Theory and Practice* [M]. Oxford: Blackwell Science Inc

Chen S, Wu G, Zhang D *et al.*, 2008. Potential refugium on the Qinghai-Tibet Plateau revealed by the chloroplast DNA phylogeography of the alpine species *Metagentiana striata* (Gentianaceae) [J]. *Botanical Journal of the Linnean Society*, **157** (1): 125—140

Clement M, Posada D, Crandall KA, 2000. TCS: a computer program to estimate gene genealogies [J]. *Molecular Ecology*, **9** (10): 1657—1659

Doyle JJ, Doyle JL, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue [J]. *Phytochemical Bulletin*, **19** (1): 11—15

Excoffier L, Laval G, Schneider S, 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis [J]. *Evolutionary Bioinformatics Online*, **1**: 47—50

Excoffier L, Smouse PE, Quattro JM, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data [J]. *Genetics*, **131** (2): 479—491

Frankham R, Ballou JD, Briscoe DA, 2002. *Introduction to Conservation Genetics* [M]. Britain: Cambridge University Press

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**: 95—98

Hamrick JL, Godt MJW, Sherman-Broyles SL, 1992. Factors influencing levels of genetic diversity in woody plant species [J]. *New Forests*, **6** (1): 95—124

Hedrick PW, Miller PS, 1992. Conservation genetics: techniques and fundamentals [J]. *Ecological Applications*, **2** (1): 30—46

Jin ZZ (金振洲), 2002. *Floristic Features of Dry-Hot and Dry-Warm Valleys in Yunnan and Sichuan* (滇川干热河谷与干暖河谷植物区系特征) [M]. Kunming: Yunnan Science and Technology Press

Jin ZZ, Ou XK, Ou PD *et al.*, 1994. A preliminary study on the floristic characteristics of seed plants in the dry-hot river valley of JinShaJiang [J]. *Acta Botanica Yunnanica* (云南植物研究), **16** (1): 1—16

Li XW, Li J, 1993. A preliminary floristic study on the seed plants from the region of Hengduan Mountain [J]. *Acta Botanica Yunnanica* (云南植物研究), **15** (3): 217—231

Liu L, 1980. *Flora of China* (Vol. 28) [M]. Beijing: Science press

Marshall D, Brown A, 1975. Optimum sampling strategies in genetic conservation [J]. *Crop Genetic Resources for Today and Tomorrow*, 53—80

Mu J, Li ZB, Li P *et al.*, 2010. A study on vegetation restoration technology of abandoned dreg site of hydropower station in the dry- hot valley areas [J]. *Journal of Basic Science and Engineering*, **18**: 245—251

Myers N, Mittermeier RA, Mittermeier CG *et al.*, 2000. Biodiversity hotspots for conservation priorities [J]. *Nature*, **403** (6772): 853—858

Nei M, Li WH, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases [J]. *Proceedings of the National Academy of Sciences of USA*, **76** (10): 5269

Nei M, Tajima F, 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data [J]. *Genetics*, **105** (1): 207

Petit RJ, 1999. Diversitégénétique et histoire des populations d'arbres forestiers [J]. Dossier d'habilitaion à diriger des recherches, Université de Paris-Sud, Université Formation de Recherche Scientifique d'Orsay, Paris, France

Rogers AR, Harpending H, 1992. Population growth makes waves in the distribution of pairwise genetic differences [J]. *Molecular Biology and Evolution*, **9** (3): 552—569

Rozas J, Sánchez-DelBarrio JC, Messeguer X *et al.*, 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods [J]. *Bioinformatics*, **19** (18): 2496—2497

Templeton AR, Sing CF, 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination [J]. *Genetics*, **134** (2): 659—669

Thompson JD, Gibson TJ, Plewniak F *et al.*, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools [J]. *Nucleic Acids Research*, **25** (24): 4876—4882

Wakeley J, 2008. *Coalescent Theory* [M]. USA: Roberts & Company

Wang W, Lu AM, Ren Y *et al.*, 2009. Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data [J]. *Perspectives in Plant Ecology, Evolution and Systematics*, **11** (2): 81—110

Wang Y, Wu JQ, Tao Y *et al.*, 2003. Natural distribution and ex situ conservation of endemic species *Myricaria laxiflora* in water-level-fluctuation zone within Three-Gorges Reservoir area of Changjiang river [J]. *Jounal of Wuhan Botanical Research* (武汉植物学研究), **21** (5): 415—422

Wright S, 1931. Evolution in Mendelian populations [J]. *Genetics*, **16** (2): 97

Wu JQ, Zhao ZE, Jin YX *et al.*, 1998. Investigation and study on the endemic plant *Myricaria laxiflora* in the Three-Gorge reservoir area [J]. *Jounal of Wuhan Botanical Research* (武汉植物学研究), **16** (2): 111—116

Young A, Boyle T, Brown T, 1996. The population genetic consequences of habitat fragmentation for plants [J]. *Trends in Ecology & Evolution*, **11** (10): 413—418

Zhang GL, Gong X, 2002. The karyotype analysis of *Anemoclema glaucifolium* and *Heteroplexis microcephala* both endimic to China [J]. *Acta Botanica Yunnanica* (云南植物研究), **24** (6): 765—768